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# VALIDATED RP-HPLC METHOD FOR ESTIMATION OF GRANISETRON IN API AND DOSAGE FORM

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**Abstract:**

*A rapid and precise Reverse Phase High Performance Liquid Chromatographic method has been developed for the validated of Granisetron, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Symmetry C18 (4.6 x 250mm, 5µm) column using a mixture of Acetonitrile and Water (50:50% v/v) as the mobile phase at a flow rate of 0.8ml/min, the detection was carried out at 285nm. The retention time of the Granisetron was 3.0 ±0.02min. The method produce linear responses in the concentration range of 20-100µg/ml of Granisetron. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.*

**Keywords:** *Granisetron, RP-HPLC, validation.*

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## INTRODUCTION:

HPLC is also called as high pressure liquid chromatography since high pressure is used to increase the flow rate and efficient separation by forcing the mobile phase through at much higher rate. The pressure is applied using a pumping system. The development of HPLC from classical column chromatography can be attributed to the development of smaller particle sizes. Smaller particle size is important since they offer more surface area over the conventional large particle sizes. The HPLC is the method of choice in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages.

1. Improved resolution of separated substances
2. column packing with very small (3,5 and 10 µm) particles
3. Faster separation times (minutes)
4. Sensitivity
5. Reproducibility
6. continuous flow detectors capable of handling small flow rates
7. Easy sample recovery, handling and maintenance [1]

### Types of HPLC Techniques

### Based on Modes of Chromatography

These distinctions are based on relative polarities of stationary and mobile phases

**Reverse phase chromatography:** In this the stationary phase is non-polar and mobile phase is polar. In this technique the polar compounds are eluted first and non polar compounds are retained in the column and eluted slowly. Therefore it is widely used technique.

**Normal phase chromatography:** In this the stationary phase is polar and mobile phase is non-polar. In this technique least polar compounds travel faster and are eluted first where as the polar compounds are retained in the column for longer time and eluted [2-5].

### Based on Principle of Separation

**Liquid/solid chromatography (Adsorption):** LSC, also called adsorption chromatography, the principle involved in this technique is adsorption of the components onto stationary phase when the sample solution is dissolved in mobile phase and passed through a column of stationary phase. The basis for separation is the selective adsorption of polar compounds; analytes that are more polar will be attracted more strongly to the active silica gel sites. The solvent strength of the mobile phase determines the rate at which adsorbed analytes are desorbed and elute. It is widely used for separation of isomers and classes of compounds differing in polarity and number of functional groups. It works best with compounds that have relatively low or intermediate polarity [6-8].

**Liquid/Liquid chromatography (Partition Chromatography):** LLC, also called partition chromatography, involves a solid support, usually silica gel or kieselguhr, mechanically coated with a film of an organic liquid. A typical system for NP LLC column is coated with ß, ß'-oxy dipropionitrile and a non-polar solvent like hexane as the mobile phase. Analytes are separated by partitioning between the two phases as in solvent extraction. Components more soluble in the stationary liquid move more slowly and elute later [9-11].

**Ion exchange:** In this the components are separated by exchange of ions between an ion exchange resin stationary phase and a mobile electrolyte phase. A cation exchange resin is used for the separation of cations and anion exchange resin is used to separate a mixture of anions [12-17].

**Size exclusion:** In this type, the components of sample are separated according to their molecular sizes by using different gels (polyvinyl acetate gel, agarose gel). ex: separation of proteins, polysaccharides, enzymes and synthetic polymers. [3,15]

**Chiral chromatography:** In this type of chromatography optical isomers are separated by using chiral stationary phase.

**Affinity chromatography:** In this type, the components are separated by an equilibrium between a macromolecular and a small molecule for which it has a high biological specificity and hence affinity. [3]

### Based on elution technique

**Isocratic separation**: In this technique, the same mobile phase combination is used throughout the process of separation. The same polarity or elution strength is maintained throughout the process.

**Gradient separation:** In this technique, a mobile phase combination of lower polarity or elution strength is followed by gradually increasing polarity or elution strength [3].

**Based on the scale of operation**

**Analytical HPLC:** Where only analysis of samples are done. Recovery of samples for reusing is normally not done, since the sample used is very low. Ex: µg quantities.

**Preparative HPLC:** Where the individual fractions of pure compounds can be collected using fraction collector. The collected samples are reused. Ex: separation of few grams of mixtures by HPLC.[4]

**Based on type of analysis**

**Qualitative analysis**: Which is used to identify the compound,detect the presence of impurities to find out the number of components. This is done by using retention time values.

**Quantitative analysis:** This is done to determine the quantity of individual or several components of mixture. This is done by comparing the peak area of the standard and sample [3].

**INSTRUMENTATION OF HPLC**

The basic liquid chromatograph consists of six basic units. The mobile phase supply system, the pump and programmer, the sample valve, the column, the detector and finally a means of presenting and processing the results.

**Mobile phase (solvent) reservoirs and solvent degassing**

The mobile phase supply system consists of number of reservoirs (200 mL to 1,000 mL in capacity). They are usually constructed of glass or stainless steel materials which are chemically resistant to mobile phase**.**

**Mobile phase**

Mobile phases in HPLC are usually mixtures of two or more individual solvents. The usual approach is to choose what appears to be the most appropriate column, and then to design a mobile phase that will optimize the retention and selectivity of the system. The two most critical parameters for nonionic mobile phases are strength and selectivity [8,17].

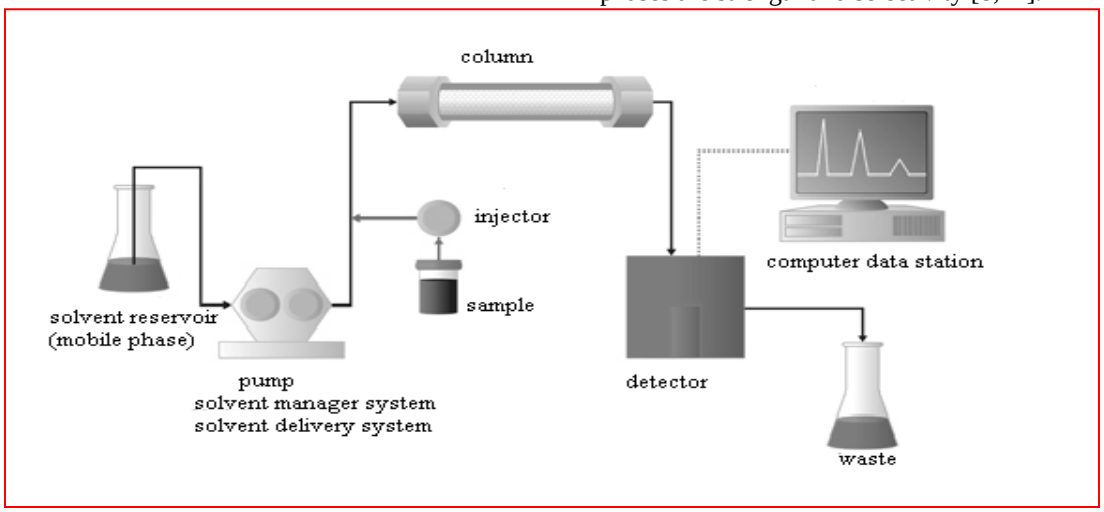


**Fig.1: Components of HPLC instrument block diagram.**

**Mobile phase preparation**

Mobile phases must be prepared from high purity solvents, including water that must be highly purified. Mobile phases must be filtered through ≤1 µm pore size filters and be degassed before use.

**Degassing of solvents**

Many solvents and solvent mixtures (particularly aqueous mixtures) contain significant amounts of dissolved nitrogen and oxygen from the air. These gasses can form bubbles in the chromatographic system that cause both serious detector noise and loss of column efficiency. These dissolved gases in solvent can be removed by the process of degassing.

Every solvent must be degassed before introduction into pump as it alter the resolution of column and interfere with monitoring of the column effluent.

Degassing is done in many ways:

1. By warming the solvents
2. By stirring vigorously with a magnetic stirrer
3. By subjecting to vaccum filtration
4. By ultra sonication (using ultrasonicator)
5. By bubbling He gas through the solvent reservoir. [8]

**Granisetron**: 1-Methyl-N-((1R,3r,5S)-9-methyl-9-azabicyclo[3.3.1]nonan-3-yl)-1H indazole-3-carboxamide Granisetron is a potent, selective antagonist of 5-HT3 receptors. The antiemetic activity of the drug is brought about through the inhibition of 5-HT3 receptors present both centrally (medullary chemoreceptor zone) and peripherally (GI tract). This inhibition of 5-HT3 receptors in turn inhibits the visceral afferent stimulation of the vomiting center, likely indirectly at the level of the area postrema, as well as through direct inhibition of serotonin activity within the area postrema and the chemoreceptor trigger zone.

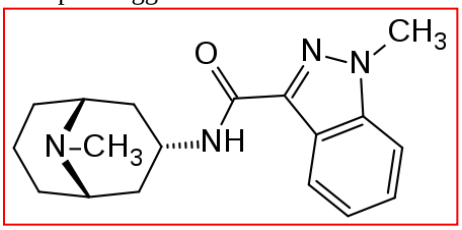


**Fig. 2: chemical structure of Granisetron**

## MATERIALS AND METHODS:

Accurately measured 500ml (50%) of HPLC Water and 500ml (50%) of HPLC Acetonitrile in to a 1000ml of volumetric flask and degassed in a digital ultrasonicator for 10 minutes**.**

**Instrumentation and Chromatographic conditions**

The analysis was performed by using Symmetry C18 column, 4.6×250mm internal diameter with 5 micron particle size column and UV detector set at 285nm nm, in conjunction with a mobile phase of Acetonitrile: Water in the ratio of 50:50v/v (pH 5 adjusted with OPA) at a flow rate of 0.8 ml/min. The retention time of Granisetron was found to be 3.008 minute. The 10µl of sample solution was injected into the system

**Preparation of standard solution:**

Accurately weigh and transfer 10 mg of Granisetron working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.6ml of the above Granisetron stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

**Mobile Phase Optimization**:

Initially the mobile phase tried was Methanol: Water, Acetonitrile: Water with varying proportions. Finally, the mobile phase was optimized to Acetonitrile: and Water in proportion 50:50 v/v respectively.

**Optimization of Column:**

The method was performed with column like Symmetry C18 (4.6×250mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 0.9ml/min flow.

**(Optimized chromatogram):**

| | |
|---|---|
| Column (4.6×250mm) 5µ | :Symmetry C18 |
| Column temperature | : Ambient |
| Wavelength | : 285nm |
| Mobile phase ratio v/v) | : Acetonitrile:Water(50:50 |
| Flow rate | : 0.8ml/min |
| Injection volume | : 10µl |
| Run time | : 6minutes |

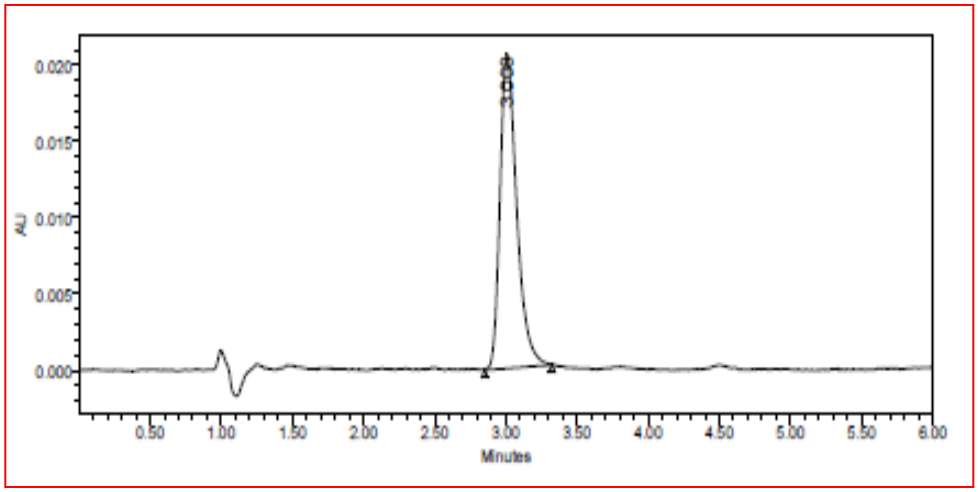


**Fig. 3: Typical chromatogram of mixture of Standard solution.**

**VALIDATION**

**PREPARATION OF MOBILE PHASE:**

**Preparation of mobile phase:**

Accurately measured 500ml (50%) of HPLC Water and 500ml (50%) of HPLC Acetonitrile in to a 1000ml of volumetric flask and degassed in a digital ultrasonicator for 10 minutes.

**Diluent Preparation:**

The Mobile phase was used as the diluent.

**Linearity**

The linearity of was obtained in the concentration ranges from 20-100 µg/ml

**Table 1: Linearity data of Grantisftron**

| Concentration Level (%) | Concentration µg/ml |
|---|---|
| 60 | 20 |
| 80 | 40 |
| 100 | 60 |
| 120 | 80 |
| 140 | 100 |

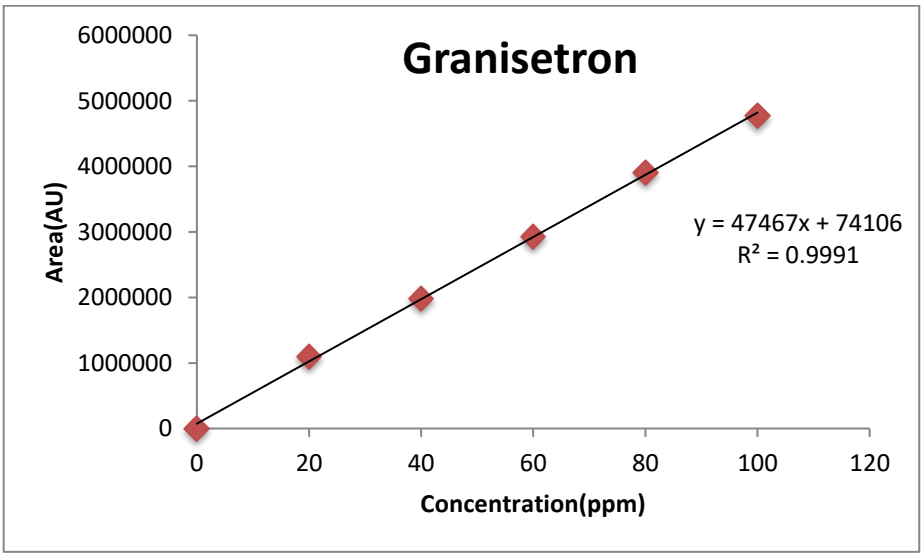


**Fig.4: calibration graph of Grantisftron**

**LINEARITY PLOT**

Linearity of detector response of assay method was found by injecting seven standard solutions with concentration ranging from 20-100 µg/mL for Grantisftron. The graph was plotted for concentration versus peak area.

**Precision**

**Repeatability**

The precision of test method was determined by preparing six test preparations using the product blend and by mixing the active ingredient with excipients as per manufacturing formula. And the relative standard deviation of assay results was calculated.

**Table 2: Results of repeatability for Grantisftron**

| S. No | Peak name | Retention time | Area (µV*sec) | Height (µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Granisetron | 2.942 | 168306 | 20744 | 7562 | 1.1 |
| 2 | Granisetron | 2.962 | 168388 | 20788 | 9981 | 1.2 |
| 3 | Granisetron | 2.963 | 168365 | 20727 | 6794 | 1.1 |
| 4 | Granisetron | 2.804 | 162052 | 21841 | 8927 | 1.2 |
| 5 | Granisetron | 2.865 | 163387 | 21947 | 7746 | 1.1 |
| **Mean** | | | 166099.6 | | | |
| **Std.dev** | | | 3121.629 | | | |
| **%RSD** | | | 1.879372 | | | |

**Accuracy**

Grantisftron tablets content were taken at various concentrations ranging from 50 % to 150 % (50 %, 75 %, 100 %, 125 %, and 150 %) to accurately quantify and to validate the accuracy. The assay was performed in triplicate. The results were shown in Table-3

**Table 3: The accuracy results for Grantisftron**

| %Concentration (at specification Level) | Peak area | Amount Added (ppm) | Amount Found (ppm) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 86774 | 30 | 30.0 | 100 | 99.6 |
| 100% | 168427 | 60 | 59.8 | 99.3 | |
| 150% | 255311 | 90 | 89.9 | 99.7 | |

**LIMIT OF DETECTION (LOD)**

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.The LOD value for Grantisftron 2.3µg µg/ml.

**Quantitation limit (LOQ)**

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a

sample which can be quantitatively determined. The LOQ values for Grantisftron 7.0 µg/ml

**ROBUSTNESS**

The robustness was performed for the flow rate 0.8 ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Balofloxacin. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard sample of Grantisftron were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor and plate count. Table 4

**Table 4: Results for Robustness of Grantisftron**

| Parameter used for sample analysis | Peak Area | Retention Time | Theoretical plates | Tailing factor |
|---|---|---|---|---|
| Actual Flow rate of 0.8mL/min | 168461 | 3.008 | 8846 | 1.12 |
| Less Flow rate of 0.7mL/min | 167261 | 4.608 | 7927 | 1.1 |
| More Flow rate of 0.9mL/min | 167651 | 3.495 | 6927 | 1.1 |
| Less organic phase (about 5 % decrease in organic phase) | 168947 | 4.609 | 8826 | 1.2 |
| More organic phase (about 5 % Increase in organic phase) | 160081 | 3.499 | 9971 | 1.1 |

## SUMMARY AND CONCLUSION:

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 285nm and the peak purity was excellent. Injection volume was selected to be 10µl which gave a good peak area. The column used for study was Symmetry $C_{18}$ because it was giving good peak.

35ºC temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Mobile phase is Water: Acetonitrile (50:50% v/v) was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Granisetron in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Granisetron was freely soluble in acetonitrile ethanol, methanol and sparingly soluble in water.

Water: Acetonitrile (50:50% v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise.

**Table 5: Summary data for Granisetron**

| Parameters | Granisetron |
|---|---|
| Retention Time (min.) | 3.008 |
| Linearity (µg/ml) | 20-100 |
| Correlation Coefficient ($r^2$) | 0.999 |
| Slope | 47467 |
| Y - intercept | 74106 |
| LOD (µg/ml) | 2.3 |
| LOQ (µg/ml) | 7.0 |
| Repeatability (% RSD) n=6 | 1.879372 |
| Intraday Precision (% RSD) n=6 | 0.285619 |
| Interday Precision (% RSD) n=6 | 0.272518 |
| Accuracy (%) | 99.6 |

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